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Absorption spectra measured for aquatic particles concentrated onto glass-fiber filters require a correction for the increase in pathlength caused by multiple scattering in the glass-fiber filter. A multiple scattering correction was calculated from optical density spectra for 48 phytoplankton cultures of seven species representing a variety of cell sizes, pigment groups, and cell-wall types. The relationship between optical density in suspensions and on filters was not wavelength-dependent. Differences between blank filters were always spectrally neutral. Small differences between relationships for single species were inconclusive. Given the absence of wavelength-dependent effects, we report a single general quadratic relationship, $OD_{susp}(\lambda) = 0.378\ OD_{filt}(\lambda) + 0.523\ OD_{filt}(\lambda)^2$ ($r^2 = 0.988$), for correcting glass-fiber filter spectra. For independent samples, the average error in predicting $OD_{susp}(\lambda)$ with this algorithm at any wavelength was 2%. Greatest errors were in spectral regions of low absorption. Absorption spectra for particles concentrated onto glass-fiber filters can be quantitatively corrected for multiple scattering within this limit. Applicability of the algorithm to field samples of varied composition was enhanced by using a large number of spectra and a range of cell types in algorithm development.

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Quantifying absorption by aquatic particles: A multiple scattering correction for glass-fiber filters

Abstract—Absorption spectra measured for aquatic particles concentrated onto glass-fiber filters require a correction for the increase in pathlength caused by multiple scattering in the glass-fiber filter. A multiple scattering correction was calculated from optical density spectra for 48 phytoplankton cultures of seven species representing a variety of cell sizes, pigment groups, and cell-wall types. The relationship between optical density in suspensions and on filters was not wavelength-dependent. Differences between blank filters were always spectrally neutral. Small differences between relationships for single species were inconclusive. Given the absence of wavelength-dependent effects, we report a single general quadratic relationship, $OD_{susp}(\lambda) = 0.378\ OD_{filt}(\lambda) + 0.523\ OD_{filt}(\lambda)^2$ ($r^2 = 0.988$), for correcting glass-fiber filter spectra. For independent samples, the average error in predicting $OD_{susp}(\lambda)$ with this algorithm at any wavelength was 2%. Greatest errors were in spectral regions of low absorption. Absorption spectra for particles concentrated onto glass-fiber filters can be quantitatively corrected for multiple scattering within this limit. Applicability of the algorithm to field samples of varied composition was enhanced by using a large number of spectra and a range of cell types in algorithm development.

Variability in the quantity and spectral quality of light absorbed by particles is a major cause of variability in light attenuation in the ocean. Unfortunately, absorption by particles is difficult to measure because suspended particles both scatter and absorb light and they are present in low concentration in much of the ocean. Yentsch (1962) pioneered a technique that increases the absorption signal to a measurable level; he concentrated particles onto a membrane filter and measured their absorption spectrum while the particles remained on the filter. Glass-fiber filters, which act as optical diffusers in analogy with Shibata's (1958) opal glass technique, are now commonly used, but quantitative use of these data requires correction for the increase in effective pathlength caused by multiple scattering within the filters. This technique does not measure absorption by a single particle but instead represents the population of particles. Multiple scattering by the glass fibers in the filter overshadows the increased potential for particle–particle interactions caused by concentration of the particles. Subtleties such as variations in specific absorption coefficients that arise from pigment packaging effects are still apparent in spectra measured by this method (e.g. Bricaud and Stramski 1990) but these variations represent the population average. Controversy regarding the best approach to the multiple scattering correction re-

mains, and published results vary (e.g. Kishino et al. 1985; Maske and Haardt 1987; Bricaud and Stramski 1990; Mitchell 1990). This note supports the use of an empirical quadratic algorithm, lacking wavelength dependence, for this correction. Agreement with Mitchell's (1990) result suggests that general application of a single algorithm may be possible, allowing use of consistent methods between different investigators.

Kiefer and SooHoo (1982) refined the filter method, minimizing loss of scattered light by placing the filter close to the detector and adding a correction for the increase in effective pathlength caused by scatter within the glass-fiber filter. This pathlength amplification factor, β, is defined as the ratio of the optical to geometrical pathlength (Butler 1962). Kiefer and SooHoo (1982) proposed that β is close to 6 for two layers of stacked glass-fiber filters (most subsequent investigators use a single layer). After correction of the pathlength with β, absorption coefficient spectra [$a_p(\lambda)$; m^{-1}] are calculated from optical density spectra (dimensionless):

$$a_p(\lambda) = \frac{2.3[\mathrm{OD}_{\mathrm{filt}}(\lambda) - \mathrm{OD}_{\mathrm{filt}}(750)]}{\beta(V/A)} \quad (1)$$

where 2.3 converts from log base 10 to log base e, V (m^3) is the volume filtered, and A (m^2) is the clearance area of the filter.

Efforts to evaluate β have increased in complexity since Kiefer and SooHoo's (1982) assumption of constancy. β has been calculated from comparisons of suspensions and filters and shown to vary with the optical density of the sample on the filter. Mitchell and Kiefer (1988) presented a nonlinear relationship between $\beta(\lambda)$ and $\mathrm{OD}_{\mathrm{filt}}(\lambda)$ for Whatman GF/C glass-fiber filters. They related $\beta(\lambda)$ for other types of filters to the equation for GF/C filters.

Kishino et al. (1985) simply calculated the pathlength amplification factor as the ratio of filter to suspension values, without considering β to vary with optical density. For three different cultures of phytoplankton and one field sample, β (averaged over 400–700 nm) ranged from 2.43 to 4.71 (Kishino et al. 1985). Kishino et al. suggested that β will vary with particle density, spectrophotometer type, and filter type; however, their presentation of data averaged over the visible light region prevents

analysis of β as a function of optical density in the manner of Mitchell and Kiefer (1988).

Maske and Haardt (1987) discussed pathlength amplification caused by the diffuse nature of the light passing through the sample and filter but made no attempt to quantify the pathlength amplification factor. In their data, specific absorption coefficients [absorption normalized to chlorophyll concentration; $a_p^*(675)$] decrease as chlorophyll loading on the filter increases; increased chlorophyll loading is equivalent to an increase in $\mathrm{OD}_{\mathrm{filt}}(675)$, which can be achieved through greater volume filtered or greater cell density in the same volume. Because average values of $\beta(\lambda)$ are between 2 and 2.5, inclusion of a pathlength amplification factor would lower their $a_p^*(675)$ by this factor. Furthermore since the nonlinear relationship between $\beta(\lambda)$ and $\mathrm{OD}_{\mathrm{filt}}(\lambda)$ is steeper at low $\mathrm{OD}_{\mathrm{filt}}(\lambda)$ (*see* Mitchell and Kiefer 1988), application of $\beta(\lambda)$ would influence $a_p^*(675)$ at low filter loads more strongly than at high filter loads. For low $\mathrm{OD}_{\mathrm{filt}}(\lambda)$, the value of $\beta(\lambda)$ will be higher and the calculated $a_p^*(675)$ will decrease by a greater amount than for high $\mathrm{OD}_{\mathrm{filt}}(\lambda)$. Application of a nonlinear $\beta(\lambda)$ would reduce the observed trend in $a_p^*(675)$ with chlorophyll loading.

Yentsch and Phinney (1989) compared spectra measured for suspensions in 10-cm cuvettes with a blank glass-fiber filter acting as an optical diffuser to spectra obtained on filters. The absorption magnitude measured with these two techniques was the same (their figure 2) so they did not apply a pathlength amplification factor to their absorption data. Methodological differences between this and other approaches may underlie the contradictory results. For example, a 10-cm cuvette with an optical diffuser on the end may not capture scattered light as efficiently as an integrating sphere or a short pathlength cuvette with a diffuser. Scattered light lost along the sides of the 10-cm cuvette may never reach the optical diffuser, making these spectra an overestimate of actual absorption and explaining the apparent absence of pathlength amplification in their system.

In comparing suspension and filter values of optical density for three algal cultures, Bricaud and Stramski (1990) found that $\beta(\lambda)$ varied significantly at low $\mathrm{OD}_{\mathrm{filt}}(\lambda)$ and observed a "hysterisis effect" where $\beta(\lambda)$ was not the same

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for identical values of $OD_{filt}(\lambda)$ within a particular spectrum. For $OD_{filt}(\lambda) > 0.2$, $\beta(\lambda)$ was less variable. They corrected their field data by means of an exponential equation for $\beta(\lambda)$ which represented an average relationship developed from their laboratory data.

Using a Perkin-Elmer Lambda 6 spectrophotometer, Mitchell (1990) determined that the acceptance angle between the sample and light detector did not affect the measured optical density, i.e. differences in optical geometry did not influence the relationship between pathlength amplification and optical density. Mitchell (1990) assumed no wavelength dependency, produced independent nonlinear equations for four filter types, and advised use of a direct relationship estimating $OD_{susp}(\lambda)$ from $OD_{filt}(\lambda)$ rather than calculation of $\beta(\lambda)$ as a function of $OD_{filt}(\lambda)$. Absorption coefficient spectra are calculated by subtracting $OD_{filt}(750)$ from $OD_{filt}(\lambda)$, estimating $OD_{susp}(\lambda)$ with the quadratic algorithm, and incorporating the volume filtered and the filter area:

$$a_p(\lambda) = \frac{2.3 OD_{susp}(\lambda)}{(V/A)}. \qquad (2)$$

Mitchell concluded that the precision of the glass-fiber filter technique is 10%, the accuracy is 15%, and variations between filter lots may affect the accuracy of this method.

Due to the inconsistency of pathlength amplification factors reported in the literature, skepticism remains regarding the validity of correcting absorption spectra measured on glass-fiber filters for multiple scattering. In order to assure the proper treatment of our own data, determine whether pathlength amplification for our spectrophotometer agrees with that published for other spectrophotometers, and directly develop an algorithm for the GF/F filters that we use exclusively in our field and laboratory sampling, we collected data in fall 1989 for determination of the pathlength amplification factor. We used a Kontron Uvikon 860 dual-beam spectrophotometer equipped with a scattered light transmission accessory. This accessory positions turbid samples close to the photomultiplier tube and provides a wide-acceptance angle for capturing scattered light.

The optical density of algal cultures in suspension was measured in 1-cm cuvettes with the side facing the light detector composed of light-diffusing opal glass. Because of their small size and low refractive index relative to water, algal cells primarily scatter in the forward direction. The opal glass and close proximity of the detector combine to capture the forward-scattered light, allowing measurement of absorption. Bricaud et al. (1983) estimated the loss due to scattering as <0.5% for a similar optical orientation. Optical density spectra for suspended particles measured in these opal glass cuvettes match those measured with an integrating sphere (Weidemann and Mitchell pers. comm.). Filtered culture medium (Millipore GS, 0.22-μm pore diameter) was used as a blank.

The optical density of concentrated cultures was measured after filtration of a subsample onto a Whatman GF/F glass-fiber filter (effective pore size, 0.7 μm) at low vacuum. Subsample volume was chosen so that the geometrical pathlength of filtered samples (volume filtered divided by the clearance area of the filter) matched the 1-cm pathlength of the cuvettes, thus $OD_{filt}(\lambda)$ differed from $OD_{susp}(\lambda)$ by the magnitude of the pathlength amplification factor. Prefiltered seawater (Millipore GS) was passed through a second GF/F filter and this wet filter was used as a blank. A baseline spectrum for two wet, blank GF/F filters was stored and the baseline-correction feature of the spectrophotometer used. A drop of filtered seawater adhered the wet filters to a Plexiglas sheet; the Plexiglas was then placed in a custom-designed holder which held it upright in the scattered transmission accessory. Thorough saturation of both filters was ensured by letting filtered seawater accumulate at the base of the filter holder; however, recent laboratory measurements indicate that optical density and spectral characteristics of the sample do not vary with water content (Cleveland unpubl.). The sample side of the filter faced the light source and the Plexiglas faced the detector. $OD_{susp}(\lambda)$ and $OD_{filt}(\lambda)$ were measured from 760 to 390 nm at 4-nm spectral bandwidth.

A comparison of blank GF/F filters within the same lot ($n = 6$) and between lots ($n = 7$) was made. The baseline was stored with two filters from the same lot, and each successive filter was compared to the same reference filter. $OD_{filt}(\lambda)$ for all "sample" blank filters was spectrally flat with respect to the reference filter.

Four of these spectra showed a slight elevation of $OD_{filt}(400)$ (0.004 relative optical density units) compared to $OD_{filt}(750)$, with a smooth, gradual slope between 400 and 750 nm. For a particulate sample with optical density of intermediate value (e.g. 0.2 optical density units), this amounts to a 2% change across the spectrum. This variability is insignificant. Spectra were always flat but optical density was not always zero. However, this difference is accounted for when $OD_{filt}(750)$ is subtracted from $OD_{filt}(\lambda)$.

Unialgal phytoplankton cultures were grown in sterile filtered, nutrient-enhanced, artificial seawater at 20°C under "cool-white" fluorescent lamps (33 μmol photons m^{-2} s^{-1}) on a 12 : 12 L/D cycle. Cultured phytoplankton were used because natural samples are not dense enough to allow direct measurement of optical density on unconcentrated samples. Algal species ranged from about 1 to 20 μm in diameter. We measured 48 pairs of spectra for seven different species: *Chaetoceros gracilis* (12 pairs of spectra), *Thalassiosira weissflogii* (11), *Amphidinium carterae* (5), *Micromonas pusilla* (2), Prasinophyte Ω 48-23 (3), *Dunaliella tertiolecta* (11), and *Synechococcus* WH7803 (4). Each species was grown to a variety of cell densities, providing a range of optical densities. For replicate samples, the C.V. ranged from 1 to 5% for suspensions and 1 to 9% for filters. Highest variation occurred at low optical density. Spectra were measured immediately after filtration. No qualitative degradation effects, such as the blue-shifted Soret peaks described by Stramski (1990), were observed and precautions against pigment degradation were not taken. As pointed out by Stramski, only certain species exhibit degradation effects, and our species are not among these. He recommends a systematic study of the effect of paraformaldehyde rather than general adoption of preservatives as a routine technique.

The respective optical densities at 750 nm were subtracted from $OD_{susp}(\lambda)$ and $OD_{filt}(\lambda)$. Spectra measured on glass-fiber filters are featureless in this region, despite the temperature dependent change that occurs in water absorption at 745 nm (Pegau and Zaneveld 1993); filters apparently do not contain enough water to exhibit this effect. Examination of spectra for all 48 suspensions measured in 1-cm cuvettes also did not reveal any features near 745

nm. We have observed temperature-dependent artifacts with 10-cm cuvettes in the Kontron spectrophotometer (*see* Pegau and Zaneveld 1993) as the reference cell warms in the sample compartment. Similar artifacts did not occur in the 1-cm cuvettes used here. The same techniques were used with both 10- and 1-cm cuvettes, indicating that the 1-cm cuvettes had sufficient time in the sample compartment to achieve a temperature differential, but the signal was not large enough to be measured.

Data analysis was restricted to the visible light region, 400–700 nm. Below 400 nm, $OD_{filt}(\lambda)$ is noisy due to low light through-put. Values of $OD_{filt}(\lambda) > 0.4$ were eliminated from the data set because our field and laboratory data are always lower than 0.4 and generally below 0.3. Restriction of the data range also provides consistency with the procedure used by Mitchell (1990).

Qualitatively, results for $\beta(\lambda)$ as a function of $OD_{filt}(\lambda)$ (not shown) resembled those of previous studies. The mean value of $\beta(\lambda)$ was 2.46, $\beta(\lambda)$ varied as an inverse function of $OD_{filt}(\lambda)$, and $\beta(\lambda)$ was more variable at low $OD_{filt}(\lambda)$. The "hysteresis effect" observed by others was not apparent in all pairs of spectra and was small when present, as opposed to the large loops shown by Bricaud and Stramski (1990).

Instead of analyzing $\beta(\lambda)$ as a function of $OD_{filt}(\lambda)$, we followed the suggestion of Mitchell (1990) and examined $OD_{susp}(\lambda)$ as a function of $OD_{filt}(\lambda)$ ($n = 13,256$). Statistical analyses were performed with International Mathematics and Statistical Libraries (IMSL). χ^2 goodness-of-fit tests indicated that a second-order polynomial represented the data better than a third-order polynomial or straight line. The resulting quadratic equation,

$$OD_{susp}(\lambda) = 0.378 \; OD_{filt}(\lambda) + 0.523 \; OD_{filt}(\lambda)^2 \qquad (3)$$

($r^2 = 0.988$), calculates $OD_{susp}(\lambda)$ from measured $OD_{filt}(\lambda)$. The 95% confidence intervals are 0.371–0.384 for the first coefficient and 0.496–0.550 for the second. An intercept was not included because absorption by the suspension should equal zero when absorption by the filtered sample equals zero. This algorithm is statistically valid only for the data range [$OD_{filt}(\lambda)$ between 0 and 0.4] over which it was developed.

Values of $OD_{susp}(\lambda)$ predicted from Eq. 3 differ by <10% from the estimates of Mitchell (1990), with the curves diverging to this maximum as $OD_{filt}(\lambda)$ increases. This result is within the expected accuracy of the method, even though coefficients in each algorithm do not fall within the 95% confidence intervals for the other. Differences in optical geometry for various spectrophotometers may influence the relationship between $OD_{susp}(\lambda)$ and $OD_{filt}(\lambda)$. Mitchell suggested that filter thickness varies with filter lot, which would also affect the relationship between $OD_{susp}(\lambda)$ and $OD_{filt}(\lambda)$. Our data were obtained before his were published; various filter lots were used and lot number was not recorded. However, as described previously, GF/F blanks from seven different lots showed no spectral differences, and differences in magnitude between various lots have no effect on the algorithm or the sample absorption after subtraction of $OD_{filt}(750)$. Despite differences in coefficients, the two algorithms give similar results, suggesting that use of a single algorithm for various spectrophotometers may be possible.

Accuracy of the algorithm was examined with 10 independent samples with $OD_{filt}(435)$ between 0.03 and 0.3. $OD_{susp}(\lambda)$ predicted from $OD_{filt}(\lambda)$ with Eq. 3 was within 2% of directly measured $OD_{susp}(\lambda)$ when averaged over all wavelengths and samples, with a maximum error of 20% for one sample in the yellow wavelength region where absorption is low. The optical densities of the filtered test samples were restricted to the range (0–0.4) used for algorithm development and typical of field samples by controlling the volume filtered.

The potential for wavelength dependence of the multiple scattering correction was examined by fitting a second-order polynomial at 30 separate wavelengths [every 10 nm, $n = 48$ except at 440 nm where two values of $OD_{filt}(440)$ exceeded 0.4]. Equations for wavelengths from 400 to 480 nm were identical. Differences between this group and other wavelengths did not follow any pattern with wavelength, as would be expected from phenomena such as wavelength-dependent scattering. Instead, these differences appeared to be an artifact of inherent differences in the range of optical density at each wavelength. Phytoplankton absorption spectra have peaks and valleys at consistent wavelengths, causing

the maximum value of $OD_{filt}(\lambda)$ to reach 0.4 at 440 nm but only 0.09 at 560 nm. As a consequence, the second coefficient, $C_2(\lambda)$, of the wavelength-specific second-order polynomials varied inversely with mean $OD_{filt}(\lambda)$ at the respective wavelengths ($r^2 = 0.71$). The first coefficient of the polynomials, $C_1(\lambda)$, varied less and did not show a similar trend with range of $OD_{filt}(\lambda)$. $C_1(\lambda)$ for only 5 of the 30 wavelengths did not fall within the 95% C.I. for all other wavelengths, indicating that spectral differences were minimal.

Wavelengths with similar ranges of $OD_{filt}(\lambda)$ (e.g. 400, 480, and 680 nm, Fig. 1) had statistically indistinguishable algorithms (t-test, $P < 0.05$) even when separated by many nanometers, such as the 280-nm difference between 400 and 680 nm. Because wavelength-specific algorithms were similar when ranges of $OD_{filt}(\lambda)$ were similar, we ascribe the apparent wavelength dependence to a statistical artifact caused by inherent differences in the range of data at the various wavelengths. No trends in the error between predicted and measured $OD_{susp}(\lambda)$ as a function of wavelength were apparent, i.e. no consistent patterns of over- or underestimation at a particular wavelength occurred, verifying the absence of wavelength-dependent trends in the algorithm.

Spectral differences attributable to scattering by glass-fiber filters, as described by Roesler and Perry (unpubl.), were not evident in these data. If present, spectral scattering by glass-fiber filters would appear as increases in apparent absorption at the blue end of the spectra or as increases in the ratio of $OD_{filt}(400)$:$OD_{filt}(675)$ compared to $OD_{susp}(400)$:$OD_{susp}(675)$. Even for spectra with low optical density, where these changes would be more obvious, no increase or flattening of $OD_{filt}(400)$ relative to $OD_{susp}(400)$ was present. Ratios of $OD_{filt}(400)$:$OD_{filt}(675)$ for all 48 pairs of spectra used in algorithm development were evenly divided between greater and lower than the corresponding ratio for the suspension. We speculate that blank filters more closely matched sample filters in our study compared to Roesler and Perry's (unpubl.) data. Comparison of GF/F filters within and between lots showed no spectral features, again discounting differences in spectral scattering between filters.

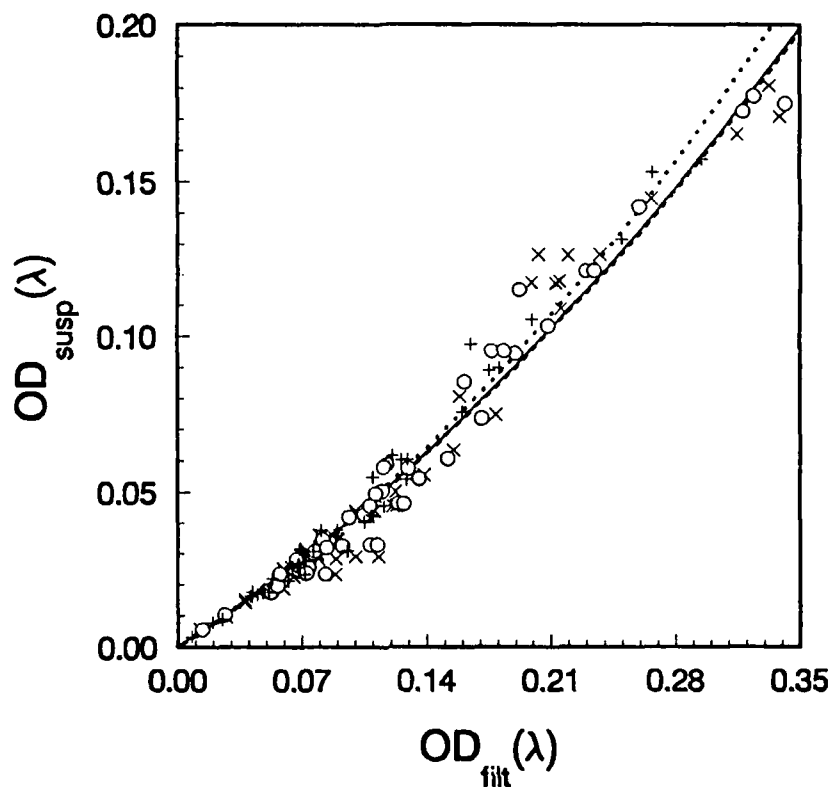Species-specific algorithms were compared

Notes

Fig. 1.   Wavelength-specific data and curve fits for $OD_{susp}(\lambda)$ as a function of $OD_{filt}(\lambda)$ at 400 nm (+, solid line), 480 nm (O, dashed line), and 680 nm (×, dotted line).

for those species with sufficient number of spectra and range of optical density to provide statistically useful algorithms. Curves for the two diatoms with siliceous frustules, *C. gracilis* (~6 μm equivalent spherical diameter) and *T. weissflogii* (~11 μm), were similar. *A. carterae* (an unarmored dinoflagellate; ~20 μm) had a steeper relationship (higher C_2), while *D. tertiolecta* (a naked chlorophyte; ~8 μm) had a shallower relationship (lower C_1 and C_2). These differences are intriguing but the data set is not diverse enough to establish trends caused by cell-wall composition or cell size. The species were chosen to produce a general algorithm rather than provide a close examination of species differences.

The analysis presented here indicated that the correction for multiple scattering in glass-fiber filters was independent of wavelength, although species independence was not conclusively shown. Development of the algorithm

from a data set including species with varied characteristics and a large number of data points increases the generality of the algorithm for use with diverse field samples. Tests on independent samples showed good predictive ability. Optical density spectra measured on glass-fiber filters can be quantitatively corrected with this algorithm.

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